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CHITOSAN DRUG DELIVERY SYSTEM

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CHITOSAN DRUG DELIVERY SYSTEM

PRIORITY

This application claims priority under 35 U.S.C. §119(e) from United States Provisional Application Serial No. 60/019,543, filed June 11, 1996.

FIELD OF THE INVENTION

The present invention relates generally to a novel drug delivery system. Specifically, the invention relates to a drug delivery system comprising a modified iron/chitosan particles and chitosan-formulated compounds which provide enhanced drug delivery via oral administration. In another aspect, the present invention relates to a chitosan matrix for oral delivery of therapeutic or prophylactic substances.

BACKGROUND OF THE INVENTION

Development of effective drug delivery systems is an integral part of research and development in the pharmaceutical industry. To date, several delivery vehicles are commercially available, including simple pills, lozenges, intravenous solutions, ointments, nasal sprays, transdermal patches, eye drops and others. For sustained release of drugs over time, delivery devices include, among others, time-release pills, osmotic pumps and compositions which support depot formation. Identification and synthesis of biopolymers has led to development of more advanced delivery systems which improve means for oral administration of drugs [Langer, *Science* 249:1527-1533 (1990)], and potentially overcome problems associated with loss of bioactivity due to degradation of drugs in the digestive tract which results in decreased availability of the drug. Of particular interest are small biopolymer particles which can trap drugs efficiently, protect drugs from the harsh environment of the digestive system, and ultimately enhance absorption in the gastro-intestinal tract.

Chitin, poly-β-(1→4) linked N-acetyl-D-glucosamine, is an biopolymer abundant in nature and has several properties which make it amenable to use as a drug delivery vehicle, including moderate solubility in diluted acid solution and high affinity for organic compounds. The deacetylated form of chitin, chitosan, has structural characteristics similar to glycosaminoglycans and shows particular promise as a exogenous matrix in reparative connective-tissue rebuilding [Muzzarelli, *et al.*, *Biomaterials* 9:247-252 (1988)]. In addition, the ability of chitosan to stimulate macrophages for tumoricidal activity and for interleukin-1 production suggests it possible use as a drug carrier for tumor patients with depressed immune systems. *Id.* These properties have led investigators to test various chitosan formulations with hopes of providing a potential drug delivery vehicle which can itself survive the gastric environment while protecting an associated drug, and which permits release of the drug over extended periods of time.

Past efforts have been directed to chitosan formulations which have been tested in limited *in vitro* assays to examine the formulations ability to maintain integrity and provide sustained drug release. Early *in vitro* studies suggested that simple dried chitosan gels may have potential use as a vehicle for sustained drug release [Miyazaki, *et al.*, *Chem. Pharm. Bull.* 29:3067-3069 (1981)]. In later studies, chitosan beads, or gels, were prepared in combination with, for example, glutaraldehyde crosslinking [Thacharodi and Ro, *Biomaterials* 16:145-148 (1995); Chandy and Sharma, *Biomaterials* 14:939-944 (1993)]; alginate [Alexakis, *et al.*, *Appl. Biochem. Biotechnol.* 50:93-106 (1995); Polk, *et al.*, *J. Pharm. Sci.* 83:178-185 (1994); Miyazaki *et al*, *Biol. Pharm. Bull.* 17:745-747 (1994); Filiprovic-Grcic, *et al.*, *Intl. J. Pharm.* 116:39-44 (1995); Bodmeier and Paeratakul, *J. Pharm. Sci.* 78:964-967 (1989)]; alginate in the presence of counterions, for example tripolyphosphate or calcium chloride [Bodmeier, *et al.*, *Pharm. Res.* 6:413-417 (1989)]; tripolyphosphate alone [Sezer and Akbuga, *Intl. J. Pharm.* 121:113-116 (1995)]; and sodium hydroxide/methanol [Chandy and Sharma,

Biomaterials 13:949-952 (1992)].

Chitosan formulations which incorporate iron appear to be limited to one instance where iron was entrapped in chitosan beads and which were then coated with either liposomes or albumin in order to modulate the rate of iron release from the beads [Chandy and Sharma, *Biomaterials* 17:61-66 (1996)]. In that formulation, iron, derived from $FeCl_3$, was the entrapped "drug" rather than an integral component of the chitosan bead itself. Moreover, entrapment of iron was effected by spraying solubilized chitosan into a sodium hydroxide/methanol solution which produced chitosan particles with a size on the order of one millimeter in diameter.

In vivo studies using chitosan beads are considerably more limited. For example, Jameela, *et al.* [*J. Biomatter. Sci. Polym. Ed.* 6:621-632 (1994)] described a chitosan/alginate composite bead which allowed sustained release of ketoprofen for three hours after sublingual delivery in rabbits. Illum, *et al.* [*Pharm. Res.* 11:1186-1189 (1994)] demonstrated enhanced absorption of insulin in rat and sheep mucosa following nasal spray delivery of insulin entrapped in chitosan. To date, however, no effective chitosan formulation useful for oral drug delivery has been identified.

Thus there exists a need in the art to develop effective oral drug delivery vehicles which survive the harsh environment of the digestive system and permit sustained release of a desired drug which would otherwise be therapeutically unavailable or have limited availability. The previously described properties of chitosan make the abundant polysaccharide an attractive candidate for such a vehicle.

SUMMARY OF THE INVENTION

In one respect, the present invention relates to a drug delivery composition comprising iron/chitosan particles or complex within which a drug can be entrapped. The composition is particularly useful for oral administration of drugs in that the particles or complex provide protection for

the entrapped drug against the harsh environment of the digestive tract, and permit drug adsorption by a route that increases the circulatory half-life of the drug. Another advantage offered by the metal/chitosan complex is a high affinity for hydrophobic organic compounds, which permits delivery of normally insoluble drugs to the circulatory system.

In a preferred embodiment, the iron/chitosan particles are less than ten microns in size. More preferred, however, are particles less than five microns in diameter, and most preferably, all particles are less than 5 nm in diameter, in order to facilitate endocytosis by cells of the intestinal mucosa.

In another aspect, the present invention provides a chitosan matrix for oral delivery of therapeutic or prophylactic substances. Substances particularly useful in the chitosan delivery system are those which are largely unable to survive the harsh environment of the digestive tract and are therefore absorbed at levels generally too low to be useful in an oral delivery system. As an oral delivery system, the invention may be utilized in various forms, such as a powder, pill, caplet, capsule, gel, liquid, liquid suspension, emulsion, elixir, syrup, and the like, as long as the manufacturing process does not result in significant changes in bead size or in properties of the beads in the composition which have the preferred size. The form may be such that the particles themselves are released over a period of time or made available all at one time. Oral compositions are preferably designed to release the active compound at the point in the gastrointestinal tract where bioavailability is maximized and substance degradation is minimized.

While oral administration is the presently preferred method of use, other routes of administration are contemplated, including for example, subcutaneous, transdermal, intramuscular, intravenous, intranasal, intrapulmonary, intrarectal, intravaginal, intraperitoneal, ophthalmic, and the like. Optimal particle size for each mode of administration will vary and is readily determined by one of ordinary skill in the art. For example, a larger particle will be more easily tolerated in an oral system than in an injectable

system, which may be constrained by the mechanical injection device and/or constraints due to capillary size.

The presently preferred method for producing the drug delivery particles involves dissolving chitosan powder in an acidic solution, preferably containing acetic acid, and sonicating the resultant mixture with concurrent addition of a metal salt, preferably ferric ammonium citrate. In order to entrap a drug in the particles, an acid stable drug formulation is added initially to the chitosan in the acidic solution prior to sonication. Preparation of the particles, however, may be effected by numerous alternative methods.

For example, any of a number of commercially available grades of chitosan may be utilized to produce the drug delivery system, as well as chitosan at various degrees of deacetylation. Chitosan may also be produced from chitin by deacetylation with alkali treatment well known in the art. Presently preferred is chitosan which is deacetylated between approximately 50-80%. More preferred is chitosan deacetylated to 60-75%.

In addition, any of a number of acidic buffers may be utilized to dissolve the chitosan powder. The concentration of the acid solution in which chitosan is initially dissolved may vary in the range of 0.1% to 15%. Most preferred, however, is 2% acid solution. Likewise, pH of the acid solution may vary within a pH range of 1.0 to 6.8, however to most preferred buffer is in a pH range of 2 to 3.

Similarly, numerous iron salts may be used as an iron source to effect particle formation during sonication including, for example, ferric chloride. Other metal ions, for example, zinc, copper, or nickel, may also be useful in forming chitosan particles. Any physiologically acceptable acid or metal salt is contemplated by the invention.

In another embodiment a drug may be administered orally in a composition comprising chitosan, a sugar, such as, but not limited to maltose, hexose, mannose, or glucose and the drug. The invention is also directed to a method for producing an oral drug delivery system, the method comprising

the steps: (a) preparing a chitosan/sugar solution, (b) dissolving the drug in an organic solvent, such as, but not limited to acetone, methanol, ethanol, or acetonitrile) (c) mixing the results of steps (a) and (b), (d) lyophilizing the mixture of step (c), and (e) reconstituting the lyophilized mixture in a suitable oral excipient for administration to a subject. The resulting lyophilized mixture may also be ground to produce a fine powder. Further, as an oral delivery system, the invention may be utilized in various forms, such as a powder, pill, caplet, capsule, gel, liquid, liquid suspension, emulsion, elixir, syrup, and the like. Still a further embodiment contemplated is a method for oral drug administration comprising administering to a patient an oral composition comprising chitosan in combination with a sugar and a drug.

Various alternative components for the drug delivery system are also contemplated. For example, pharmaceutically acceptable oils such as, but not limited to canola oil, corn oil, peanut oil, olive oil, vegetable oil, mineral oil and the like or lipid compositions may be included in the chitosan/drug matrix, either prior to, during, or after the sonication step. Addition of lipids may necessitate addition of any of a number of physiologically acceptable surfactants, depending on the physical characteristics of the drug to be incorporated. Oils or lipids may provide additional protection for the entrapped drug as it traverses the gastro-intestinal tract, slow the release of the therapeutic or prophylactic substance from the chitosan matrix, or improve absorption in the intestines. Oils or lipids as described above may also be included to form emulsions which can then be sprayed onto a surface, dried, collected and compressed into a capsule or tablet. As another additional component, gelatin may be included as an agent to fix the chitosan matrix with the entrapped substance; the fixed therapeutic or prophylactic substance-containing particles can then be collected for delivery in a capsule form.

Sonication is performed to the extent that the resulting chitosan particles are less than ten microns in diameter, and preferably less than five microns in diameter. Most preferably sonication is continued until particles

are less than about five nanometers in diameter. The duration and power of sonication can vary as long as particles of a preferred size are obtained. In addition, any type and model of sonicator can be utilized to produce the chitosan particles, again, provided that the resultant particles are of a preferred size. For example, either probe style or water bath types of sonicator are adaptable to producing the drug delivery system. Depending on the power of the sonicator, the duration of sonication may vary from one to ten minutes. A currently preferred sonicator is a Branson Sonifier Model 250 used at approximately 60% full strength for one to three minutes in duration.

As an alternative to use of a sonicator, the drug delivery system may also be produced using various types of homogenizer, emulsifier, fluidizer and the like.

For physiological delivery of therapeutic or prophylactic substance, the preferred method for producing the chitosan matrix includes dissolving chitosan in an acidic solution, to which a therapeutic or prophylactic substance is then added. An aliquot of the mixture is withdrawn, mixed with a pharmaceutically acceptable oil, and sonicated. The resulting chitosan matrix is particularly useful for oral delivery of therapeutic or prophylactic substances, for example, hormones (such as but not limited to insulin, progesterone, estrogen, testosterone, glucocorticoids, mineralocorticoids, growth hormone), cytokines (such as, but not limited to interleukins, lymphokines, monokines), chemokines, hematopoietic factors (such as, but not limited to erythropoietin) and other therapeutic or prophylactic substances, polypeptides or proteins which are not ordinarily physiologically active following oral delivery. Proteins or polypeptides which are useful in the system may be native and purified from naturally occurring sources, recombinantly produced, or chemically synthesized.

Variations in the method for producing the drug delivery system are also comprehended by the invention. For example, chitosan particles can be prepared by the preferred method but in the absence of a drug. Once

prepared in this manner, the chitosan particles can then be mixed with a solution of a desired drug, after which the mixture can be delivered as described. Alternatively, chitosan particles can be prepared, again in the absence of a drug, the particles lyophilized, and subsequently rehydrated in a solution containing a drug.

DESCRIPTION OF THE DRAWINGS

Figure 1. sets forth the *in vitro* release rates of bromthymol blue (BTB) from iron (Fe), chitosan (Ch), or iron/chitosan particle complexes.

Figure 2. shows the bioavailability of progesterone after oral administration of progesterone/iron/chitosan particles.

Figure 3. sets forth the bioavailability of progesterone after oral administration of chitosan-formulated progesterone as compared to micronized progesterone.

Figure 4. sets forth data illustrating blood glucose levels in streptozotocin-treated animals after oral administration of either a insulin/chitosan composition or a insulin/PBS composition.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is illustrated by the following examples relating to the preparation and use of an iron/chitosan drug delivery composition and chitosan/protein drug delivery systems. Example 1 describes preparation of iron/chitosan particles. Example 2 illustrates *in vitro* retention and release of a compound in and from iron/chitosan particles. Example 3 shows *in vivo* delivery of a compound by iron/chitosan particles after a set period of time. Example 4 describes *in vivo* release of a compound by iron/chitosan particles or release of a chitosan-formulated compound as a function of time. Example 5 describes use of a chitosan matrix to orally administer insulin.

Example 1

Preparation of Iron/Chitosan Particles

A 1% SeaSanMer N2000 grade chitosan (CTC Organics, Atlanta, GA) solution was initially prepared by dissolving powdered chitosan in 2% acetic acid and the solution was autoclaved. To prepare iron/chitosan particles, a 1 to 5 ml chitosan solution was sonicated for one to three minutes while 0.2-0.3 ml of a 4% ferric ammonium citrate stock solution/ml of chitosan solution was added dropwise. The stock ferric ammonium citrate solution was initially prepared in water. The end result of these steps was in a very fine suspension of chitosan particles. Typically, the density of the chitosan particles was approximately 0.3 g wet weight per ml chitosan solution, or approximately 18 mg lyophilized solid per reconstituting ml buffer. Particles in the suspension were measured on a periodic basis using a micrometer and found to range from about 2 to about 10 microns in diameter. In order to incorporate small molecules in the iron/chitosan particles, the above procedure was modified as described below.

Example 2

In vitro Drug Release

In order to initially determine release rate from a small molecule entrapped in chitosan/iron particles produced as described above, an *in vitro* dialysis assay was performed as described below.

A. Preparation of Iron/Chitosan Particles Containing Bromthymol Blue

A 5 mg/ml solution of bromthymol blue (BTB) (Sigma, St. Louis, MO) was prepared in water and an equivalent of 200 μg BTB was mixed thoroughly with 500 μl iron/chitosan solution prepared as described in Example 1. Alternatively, an equivalent of 400 μg BTB per milliliter of 1% chitosan solution was mixed. The resulting mixture was sonicated three minutes while 0.2 to 0.4 ml of 4% ferric ammonium citrate in water solution

was added dropwise during sonication, which produced orange colored particles comprising bromthymol blue entrapped in the chitosan beads.

B. *In Vitro* Release of Bromthymol Blue From Iron chitosan Particles

In order to determine the rate at which the BTB was released to the dialysis buffer, the iron/chitosan particles containing bromthymol blue prepared as described above were placed individually in dialysis bags having a molecular weight cutoff of 12 kD and each bag immersed in 45 ml phosphate buffered saline (PBS) in a 50 ml conical tube. The tube was placed on an end to end shaker and release rate of the bromthymol blue into the buffer was measured over time by absorption at 595 nm with a Spectronic-20. Several controls were run in parallel: one containing an equal amount of bromthymol blue alone in a dialysis bag; another containing bromthymol blue mixed (but not incorporated as described in section A above, but still resulting in a uniform orange solution) with chitosan; and another bag containing bromthymol blue mixed with ferric ammonium citrate solution.

The release rates are shown in Figure 1. It can be seen from the graph that the dialysis bag containing bromthymol blue entrapped in iron/chitosan particles release the bromthymol blue at a much slower rate than the bags containing bromthymol blue alone, bromthymol blue mixed with ferric ammonium citrate, or bromthymol blue mixed with chitosan. The relatively slow release from the iron/chitosan particles is observed in the initial stages of incubation and the slow release rate becomes considerably more pronounced after longer periods of incubation. These data indicate an ability of the iron/chitosan particles to significantly retain the initially trapped molecules and to initially release the molecules at a slow rate.

Example 3

In vivo Drug Release

In view of the *in vitro* results presented in Example 2 above, experiments were designed in order to determine if the iron/chitosan particles were also capable of providing slow release of a substance *in vivo*. A water soluble fungicide, nystatin, was chosen for the initial studies.

A. Preparation of Iron/Chitosan Particles Containing Nystatin

A 2 mg/ml suspension of nystatin (Sigma) was prepared in distilled water and 100 μl was mixed thoroughly with 200 μl of the chitosan solution prepared as described in Example 1. The resulting mixture was sonicated for two to three minutes while 4% ferric ammonium citrate in water was added (100 μl/ml chitosan:nystatin mixture) producing yellowish particles. The suspension was centrifuged, the supernatant removed by aspiration, and the particles resuspended in 200 μl phosphate buffered saline (PBS). Following centrifugation the supernatant was clear, suggesting a high degree of drug incorporation, as nystatin is generally insoluble in water and gives a fine particulate appearance in aqueous solution. Drug incorporation in the chitosan particles was determined by HPLC (Waters) on a C-18 column and by extracting entrapped nystatin from the particles with cold methanol.

B. Serum Levels of Nystatin Following Oral Administration of Nystatin Iron/Chitosan Particles

A test group of adult female mice were orally administered iron/chitosan particles containing nystatin with a feeding needle fitted on 1 ml syringe. A first control group of mice was fed nystatin suspended in PBS and a second control group was fed a chitosan suspension mixed with nystatin to which 5 μl glutaraldehyde was added to obtain a gel. Each mouse received an equivalent of approximately 4 mg nystatin in a volume of 200-400 μl.

The mice were bled at 0, 1 and 5 hr interval through retro-orbital

puncture. The blood was allowed to clot and serum was separated. After extracting the serum with cold methanol, the serum levels of nystatin were measured using HPLC as described above

Blood nystatin concentration values at five hours are shown in Table 1 below; nystatin was not detectable at zero and one hour after administration. The mice that received nystatin entrapped in iron/chitosan particles produced higher serum nystatin levels after five hours as compared to mice that received nystatin alone or merely mixed with chitosan. In addition, comparison of serum nystatin levels between groups in which mice received either oral or intraperitoneal administration of nystatin in iron/chitosan particles, it was observed that mice receiving oral administration had greater than 150% more serum nystatin than the intraperitoneal group. This result suggests that association with chitosan provided a higher level of available drug.

Table 1 - Blood Nystatin Concentration Following Oral Administration Using Various Delivery Systems

FORMULATION	CONCENTRATION (μg/ml)
Nystatin in PBS	0.938
Nystatin mixed with Chitosan and Glutaraldehyde	0.338
Nystatin in Iron/Chitosan Particles	2.964
Nystatin in Iron/Chitosan Particles*	1.787

*This formulation is identical to the formulation immediately above, except that administration was intraperitoneal.

Example 4

In vivo Drug Release

In order to further evaluate the effectiveness of iron/chitosan particles for *in vivo* delivery and release of a drug, various groups of rats were orally administered progesterone prepared as described below (A-B). This experiment differed from that in Example 3 in that the effect of water solubility of the entrapped drug was also assessed. Further, in order to evaluate the effectiveness of a chitosan-formulated compound to be delivered and released *in vivo*, two groups of rats were orally administered progesterone prepared as described below (C-D).

A. Preparation of Iron/Chitosan Particles Containing Progesterone

Progesterone (Sigma Chemical Co., St. Louis, MO) was dissolved in dimethyl sulfoxide (DMSO; Sigma) to a final concentration of 10 mg/ml and 1 ml of the solution was mixed with an equal volume of 1% iron/chitosan containing 0.1% Tween 20 and 1 ml mineral oil. The resulting emulsion was sonicated one to three minutes while 50 μl ferric ammonium citrate stock solution was added dropwise. Particles in the resulting solution were measured under the microscope and were determined to range in size from two to ten microns. Rats receiving administered this preparation were designated Group 1.

Control preparations included: progesterone suspended in PBS and the resulting was suspension sonicated (administered to Group 2); progesterone suspended in PBS to which ferric ammonium citrate was added during sonication (administered to Group 3); and a suspension of progesterone without ferric ammonium citrate (administered to Group 4).

In addition to the above controls, two other preparations comprising water soluble progesterone were utilized. In a water soluble form, the steroid is trapped in 2-hydroxy propyl-β-cyclodextrin and the water soluble form is commercially available from Sigma (St. Louis MO). In a first preparation of

water soluble progesterone, a 10 mg/ml steroid solution was mixed with 1% chitosan suspension (at a ratio of 10 mg progesterone equivalent/ml chitosan solution), and the resulting mixture was sonicated. This preparation was administered to rats in Group 5. The second preparation comprising water soluble progesterone was prepared similar to the first except that 50 μl 4% ferric ammonium citrate was added during sonication to obtain iron/chitosan particles with entrapped steroid. This preparation was orally administered to rats in Group 6.

B. Bioavailability of progesterone after oral administration of progesterone iron chitosan micro particles

Adult female rats (ovaries removed) weighing between 300 and 350 grams were separately administered each of the various progesterone formulations described in section (A) with each dosage containing the equivalent of 1 mg progesterone/rat. Dosages were administered orally with a feeding needle attached to a 1 ml syringe. The rats were bled by retro-orbital puncture at 0, 2, 4, and 20 hr after administration and serum progesterone levels were measured by radioimmunoassay (RIA) using a Coat-A-Count Progesterone solid phase radioimmunoassay kit (Diagnostic Product Corporation, Los Angeles, CA).

The results are shown in Figure 2. Serum progesterone levels were found to be highest in the Group 1 rats which were administered insoluble progesterone in iron/chitosan particles, with the highest levels detected four hours after administration. Similarly high levels (particularly four hours after administration) were also detected in animals from Group 6 which were administered water soluble progesterone entrapped in iron/chitosan. While rats which received progesterone in several of the other formulations showed highest serum progesterone levels 2 hours after administration, the level of detectable progesterone reduced to near control levels at four hours. Based on a generally recognized short half-life for progesterone, this observation suggests either a differential release rate for progesterone in the various

formulations, or a different route of absorption for the steroid (either native or water soluble) entrapped in the iron/chitosan particles.

C. Preparation of Chitosan-Formulated Progesterone

200 mg of progesterone (Sigma Chemical Co., St. Louis MO) were dissolved in 5 ml of acetone (reagent grade; Fisher Scientific, Pittsburgh, PA) while 0.3 g of maltose (Fisher Scientific) were dissolved in 10 ml of a 2% chitosan solution (prepared by dissolving powdered SeaSanMer N2000 Grade Chitosan (CTC Organics, Atlanta, GA) in 0.25 M citric acid, (Fisher Scientific)). 5 ml of the progesterone/acetone solution were mixed with 5 ml of the chitosan/maltose solution. The resulting mixture was rapidly frozen in liquid nitrogen and lyophilized. After lyophilization, the weight equivalent of progesterone in the ground mixture was calculated (usually 3.03 to 3.05 mg of the fine powder = 1 mg of progesterone alone). The resulting sponge-like dried chitosan-formulated progesterone may also be ground into a fine powder. The resulting lyophilized mixture was reconstituted in deionized water for oral administration to test animals. As discussed previously, the invention may also be utilized in various forms, such as a powder, pill, caplet, capsule, gel, liquid, liquid suspension, emulsion, elixir, syrup, and the like.

D. Bioavailability of progesterone after oral administration of progesterone chitosan micro particles

Adult female rats (with ovaries removed) weighing between 300 and 350 grams were divided into two treatment groups (7 animals per group). Animals receiving the reconstituted chitosan-formulated progesterone, as described in (C), were designated Group A (test group), while animals receiving micronized (powdered form) progesterone were designated Group B (control group). Dosages, which were equivalent to 5mg progesterone/rat, were administered orally with a feeding needle attached to a 1 ml syringe. The rats were bled via tail at 0, 1, 5, 24, and 48 hrs after administration and serum progesterone levels were measured by radioimmunoassay (RIA) using

a Coat-A-Count Progesterone solid phase radioimmunoassay kit (Diagnostic Product Corporation, Los Angeles, CA).

Results, which are shown in Figure 3, indicate a significantly higher serum level of progesterone in animals receiving the chitosan-formulated progesterone mixture as compared to the control animals (micronized progesterone) at 1 hour and 48 hours post-administration (p-0.013) and 24 post-administration (p=0.001).

Example 5

In Vivo Protein Drug Release

In view of the results which demonstrated the usefulness of a chitosan matrix for physiological delivery of drugs, the ability of chitosan to deliver proteins via the same oral administration route was investigated.

A. Preparation of Chitosan Particles Containing Insulin

A 1% chitosan solution was prepared in 2% acetic acid (pH 3.9) as described in Example 1, above, except that no ferric ammonium citrate was added to the mixture. A 1 ml aliquot of the chitosan solution was combined with 12 mg bovine pancreatic insulin (Sigma, approximately 300 units) and the resulting solution was mixed well. A second 1 ml aliquot of the chitosan/acetic acid solution containing 0.1% polyoxyethylenesorbitan monolaurate (Tween 20) (Sigma) was added and the mixture vortexed. A 1 ml aliquot of this final solution was removed and added to 1.5 ml Canola oil (Hunt-Wesson, Fullerton, CA) and the mixture sonicated for one minute. The final insulin concentration in the resulting cloudy mixture was 60 units/ml.

As a control, 6 mg insulin (approximately 154 U.S.P. units) was added to 1 ml PBS and the solution mixed well. Canola oil (1.5 ml) was added to the solution and the resulting mixture sonicated for 1 minute. The resulting control solution, like the test solution above, appeared as a white emulsion-like mixture, however, the control mixture tended to separate into layers while

the test solution appeared as a stable emulsion.

B. Insulin Bioavailability From Oral Delivery in Normal Animals

Two groups of three rats were used to determine the availability of insulin following oral delivery in the chitosan matrix prepared as described above. In the assay protocol, each rat was fed 0.3 ml of a 0.625 g/ml glucose solution. Five minutes later each rat was fed 0.3 ml of either the insulin/chitosan mixture or the insulin control mixture. Blood was drawn from the tail vein of each rat at 0, 30, 60, and 120 minutes after administration of insulin, and blood glucose levels were determined using an ExacTech blood glucose testing system (MediSense, Waltham, MA).

The results are presented in Table 2 which indicate that insulin delivered orally in the chitosan matrix was able to cause a transient decrease in blood glucose levels that was not detected when insulin was orally delivered alone. These results, however, did not provide any information as to whether the observed drop in blood glucose was cause by absorbed chitosan alone.

Table 2
Blood Glucose Levels Following Insulin Administration

TIME (minutes)	GLUCOSE CONCENTRATION (mg/dl ± SD)	
	Control	Test
0	84 ± 7	90 ± 9
30	119 ± 18	50 ± 5
60	114 ± 18	63 ± 3
120	109 ± 11	106 ± 34

C. Insulin Bioavailability From Oral Delivery

In Normal and Streptozotocin-Treated Animals

In order to determine if the previously observed decrease in blood glucose resulted from an increase in bioavailability of insulin or from increased absorption of chitosan itself, the following experiments were performed using both normal rats and rats induced into a diabetic state by administration of streptozotocin (Sigma, St. Louis, MO) [Rakieten, *et al.*, *Cancer Chemotherapy Reports* **29**:91-98 (1963)]. Briefly, diabetes was induced in rats by intravenous administration of streptozotocin dissolved in normal saline buffered with anticoagulant acid citrate dextrose (ACD) solution (1:50 dilution of ACD with 0.9% NaCl). The final streptozotocin concentration of the solution was 20 mg/ml, pH 5.0. The diluted solution was sterilized by filtration through a 0.22 micron filter and used within 10 to 15 minutes of preparation. Rats were anesthetized with halothane and administered a single intravenous injection via the tail vein at a dosage of approximately 50 mg/kg body weight. Typically, prior to administration, blood glucose levels were in the range of 80 to 110 mg/dl blood. One day after administration, blood glucose levels were typically elevated to more than 600 mg/dl, and six days after administration, blood glucose levels were typically 350-450 mg/dl.

Rats were administered bovine insulin in the oral drug delivery system described in (A) and resultant changes in blooa glucose was determined. As controls in various experiments, rats from each group were also orally administered: (i) insulin in PBS, (ii) insulin prepared as in (A) above without addition of chitosan; and (iii) the preparation described in (A) above except that no insulin was added. As an additional positive control, bovine insulin in PBS was administered intramuscularly.

In the initial experiment, 18.4 units of insulin prepared as described in (A) suppressed blood glucose levels for almost 2 hours, while intramuscular injection of 0.26 units of insulin depressed blood glucose for almost one hour. When chitosan was omitted from the oral delivery system, or when insulin

was orally administered in PBS alone, no change was observed in blood glucose levels.

In subsequent experiments (see Figure 3), 18.4 units of insulin prepared as described above in (A) suppressed blood glucose levels for over 2.5 hours. Animals treated with insulin in PBS saw no change in blood glucose levels.

D. Oral Delivery of Insulin in Treating Diabetes

The previous results indicated that insulin delivered in chitosan via an oral route was capable of suppressing blood glucose levels. In order to assess the ability of orally delivered insulin to treat diabetes, an animal model was utilized wherein rats were induced into a diabetic state by intravenous administration of streptozotocin as discussed above. The onset of diabetes was monitored by measuring blood glucose levels. When sustained blood glucose was measured above 300 mg/dl, the animals were orally administered insulin in the following experiment.

Insulin prepared as described in (A) above was administered to a group of rats and blood glucose levels were measured and compared to the levels in a group administered insulin in PBS. In each group, 18.4 units of insulin were provided. In rats fed only insulin in PBS, no change in blood glucose levels were detected. In rats fed insulin in chitosan, however, blood glucose levels were suppressed over the course of the first three hours which the rats were monitored. These data indicate that the oral delivery of insulin in a chitosan matrix is useful for treating diabetes in an animal model, suggesting usefulness of the same drug delivery system for treating diabetes in humans.

Finally, the above described methods and formulations may be used with other drugs and therapeutic or prophylactic substances. Such drugs or substances may be (but are not limited to) those that are difficult to place in solution such as many psychoactive drugs (*e.g.* clozapine) or hormones (*e.g.* insulin, progesterone, estrogen, testosterone, glucocorticoids,

mineralocorticoids, growth hormone), cytokines (*e.g.*, interleukins, lymphokines, monokines), chemokines, hematopoietic factors (*e.g.*, erythropoietin) or other therapeutic or prophylactic substances.

Numerous modifications and variations in the invention as set forth in the above illustrative examples are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the invention.

WHAT IS CLAIMED:

1. An oral drug delivery composition comprising a chitosan-iron complex and a drug.

2. The composition according to claim 1 which is insoluble.

3. The composition according to claim 1 which is stable in acidic pH.

4. The composition according to claim 1 wherein the drug is progesterone.

5. A method for drug delivery comprising administering to a patient an oral composition comprising a chitosan-iron complex and a drug.

6 The method according to claim 5 wherein the composition is insoluble.

7. The method according to claim 5 wherein the composition is stable in acidic pH.

8. The method according to claim 5 wherein the drug is progesterone.

9. A method for producing an oral drug delivery composition, the method comprising the steps of:

a) dissolving chitosan and a drug in an acidic solution;

b) sonicating the mixture while adding a ferric salt solution thereby forming iron/chitosan/drug particles; and

c) collecting the particles.

10. The method according to claim 9 wherein the acidic solution is an acetic acid solution.

11. The method according to claim 9 wherein the acetic acid solution is in a concentration range of 0.1 % to 15.0%.

12. The method according to claim 9 wherein the acidic solution is a 2% solution.

13. The method according to claim 9 wherein the acidic solution is in a pH range of 1.5 to 6.5.

14. The method according to claim 9 wherein the acidic solution is in a pH range of 3.9 to 4.2.

15. The method according to claim 9 wherein the iron salt is ferric ammonium citrate.

16. The method according to claim 9 wherein the desired drug is progesterone.

17. An oral drug delivery composition prepared by the method of any of claims 9 through 16.

18. A method for oral delivery of a therapeutic or prophylactic substance to a patient comprising a composition comprising chitosan, pharmaceutically acceptable oils and said therapeutic or prophylactic substance.

19. The method according to claim 18 wherein the therapeutic or prophylactic substance is selected from a group consisting of hormones, cytokines, chemokines, and growth factors.

20. The method according to claim 18 wherein the therapeutic or prophylactic substance is insulin.

21. The method according to claim 18 wherein the oil is chosen from the group consisting of canola oil, corn oil, peanut oil, olive oil, vegetable oil, and mineral oil.

22. A method for producing a composition for oral administration of a therapeutic or prophylactic substance to an animal, the method comprising the steps of:

a) preparing a mixture of chitosan and said therapeutic or prophylactic substance;

b) adding pharmaceutically acceptable oils to form a mixture; and

c) administering the mixture orally to the animal.

23. The method according to claim 22 wherein the therapeutic or prophylactic substance is selected from a group consisting of hormones, cytokines, chemokines, and growth factors.

24. The method according to claim 22 wherein the therapeutic or prophylactic substance is insulin.

25. The method according to claim 19 wherein the oil is chosen from the group consisting of canola oil, corn oil, peanut oil, olive oil, vegetable oil, and mineral oil.

26. A drug delivery composition for oral delivery of a therapeutic or prophylactic substance produced by the method of any of claims 22 through 25.

27. An oral drug delivery composition comprising chitosan, a sugar and a drug.

28. The composition according to claim 26 which is insoluble.

29. The composition according to claim 26 which is stable in acidic pH.

30. The composition according to claim 26 wherein the sugar is maltose.

31. The composition according to claim 26 wherein the drug is progesterone.

32. A method for oral drug administration comprising administering to a patient an oral composition comprising chitosan in combination with a sugar and a drug.

33. The method according to claim 32 wherein the composition is insoluble.

34. The method according to claim 32 wherein the composition is stable in acidic pH.

35. The method according to claim 32 wherein the sugar is maltose.

36. The method according to claim 32 wherein the drug is progesterone.

37. A method for producing an oral drug delivery composition, the method comprising the steps:

(a) preparing a chitosan/sugar solution;

(b) dissolving the desired drug in a organic solvent;

(c) mixing the results of steps (a) and (b);

(d) lyophilizing the mixture of step (c); and

(e) reconstituting the lyophilized mixture in a suitable oral excipient for administration to a subject.

38. The method according to claim 37 wherein the lyophilized mixture is ground to a fine powder.

39. The method of claim 37 wherein the organic solvent is acetone.

40. The method of claim 37 wherein the organic solvent is ethanol.

41. The method of claim 37 wherein the sugar is maltose.

42. The method of claim 37 wherein the drug is progesterone.

43. An oral drug delivery composition prepared by the method of any of claims 37 through 42.

FIGURE 1

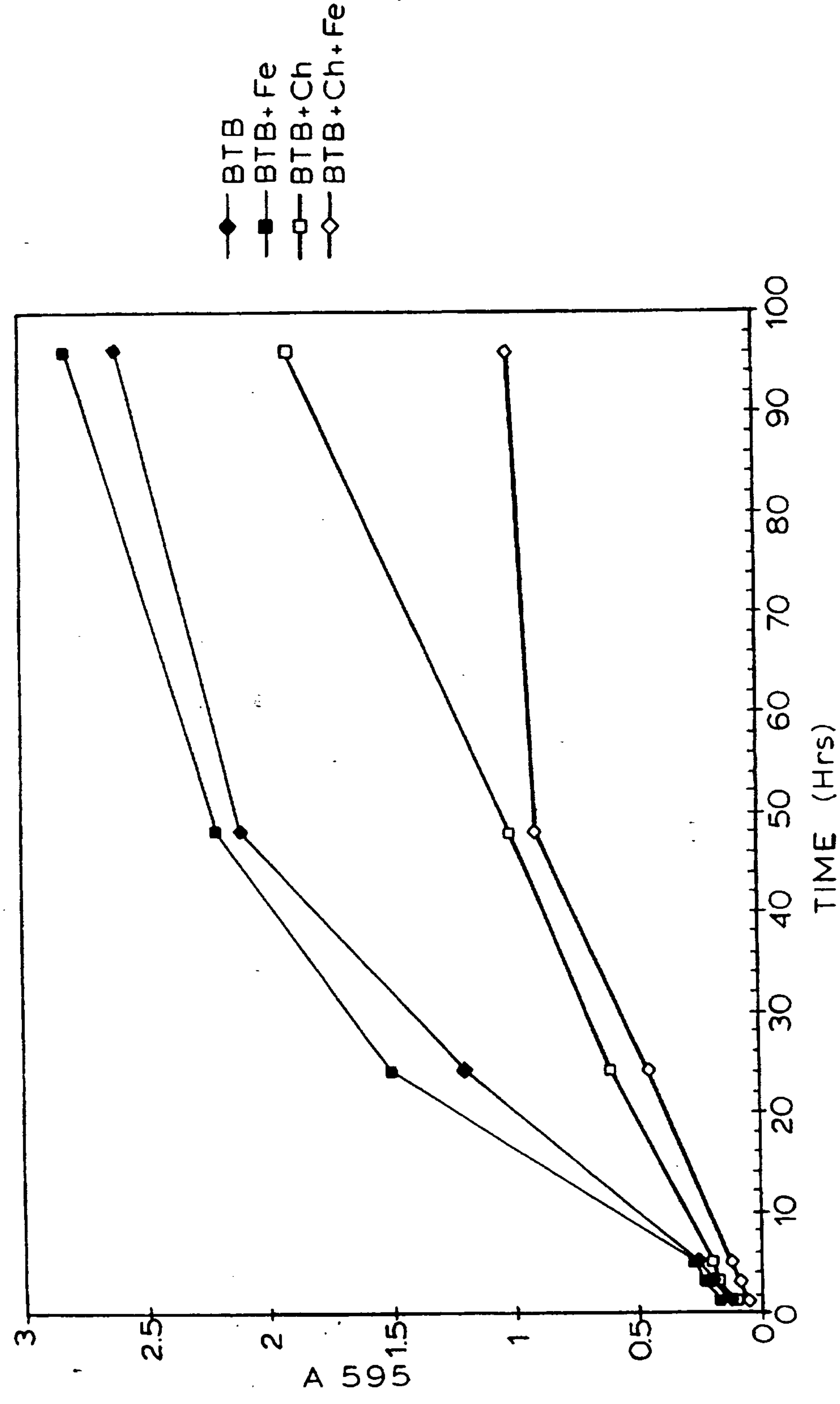

BTB
BTB+Fe
BTB+Ch
BTB+Ch+Fe
A 595
TIME (Hrs)
0
0.5
1
1.5
2
2.5
3
10
20
30
40
50
60
70
80
90
100

FIGURE 2

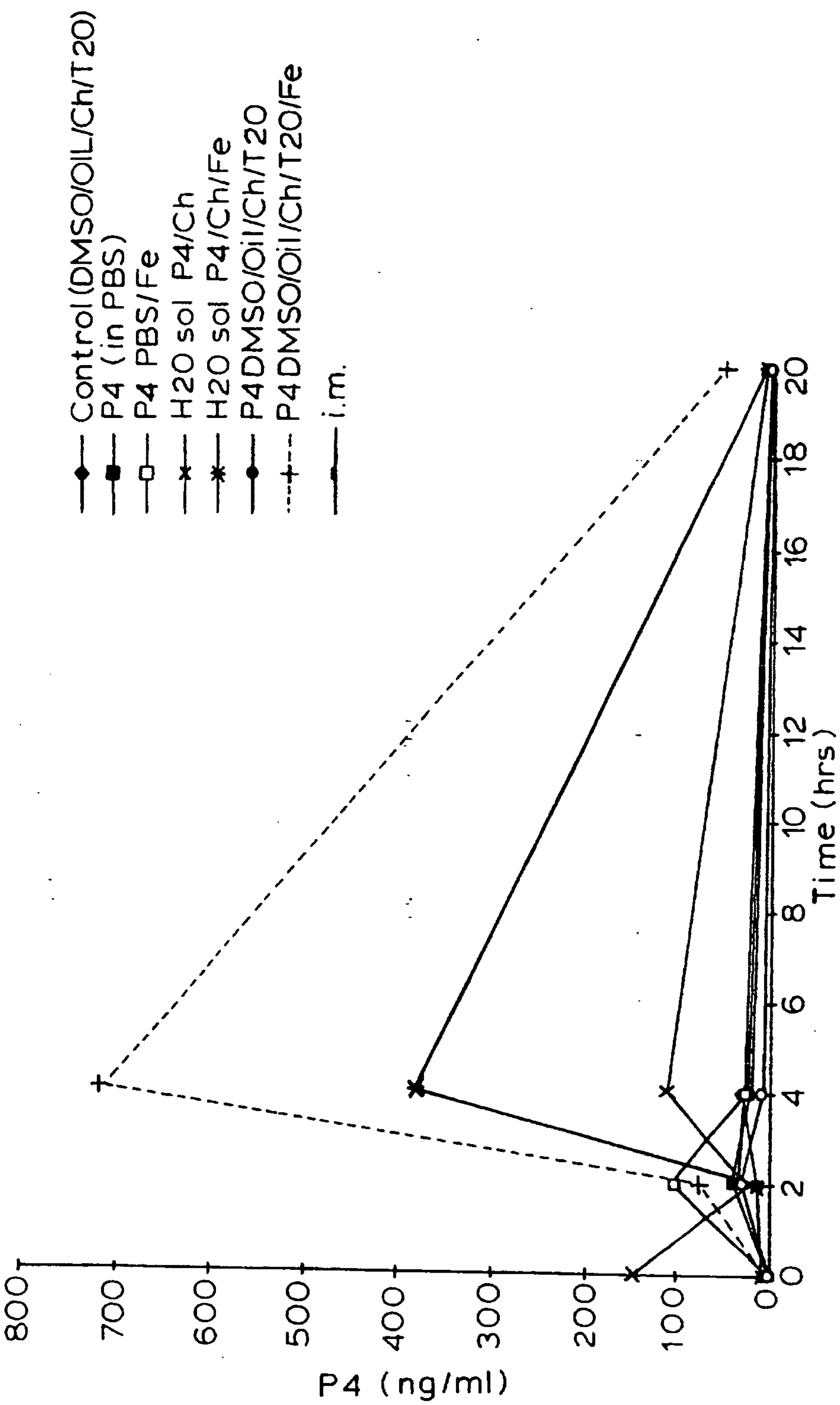

Control(DMSO/OIL/Ch/T20)
P4 (in PBS)
P4 PBS/Fe
H2O sol P4/Ch
H2O sol P4/Ch/Fe
P4DMSO/Oil/Ch/T20
P4DMSO/Oil/Ch/T20/Fe
i.m.
P4 (ng/ml)
800
700
600
500
400
300
200
100
0
Time (hrs)
0
2
4
6
8
10
12
14
16
18
20

FIGURE 3

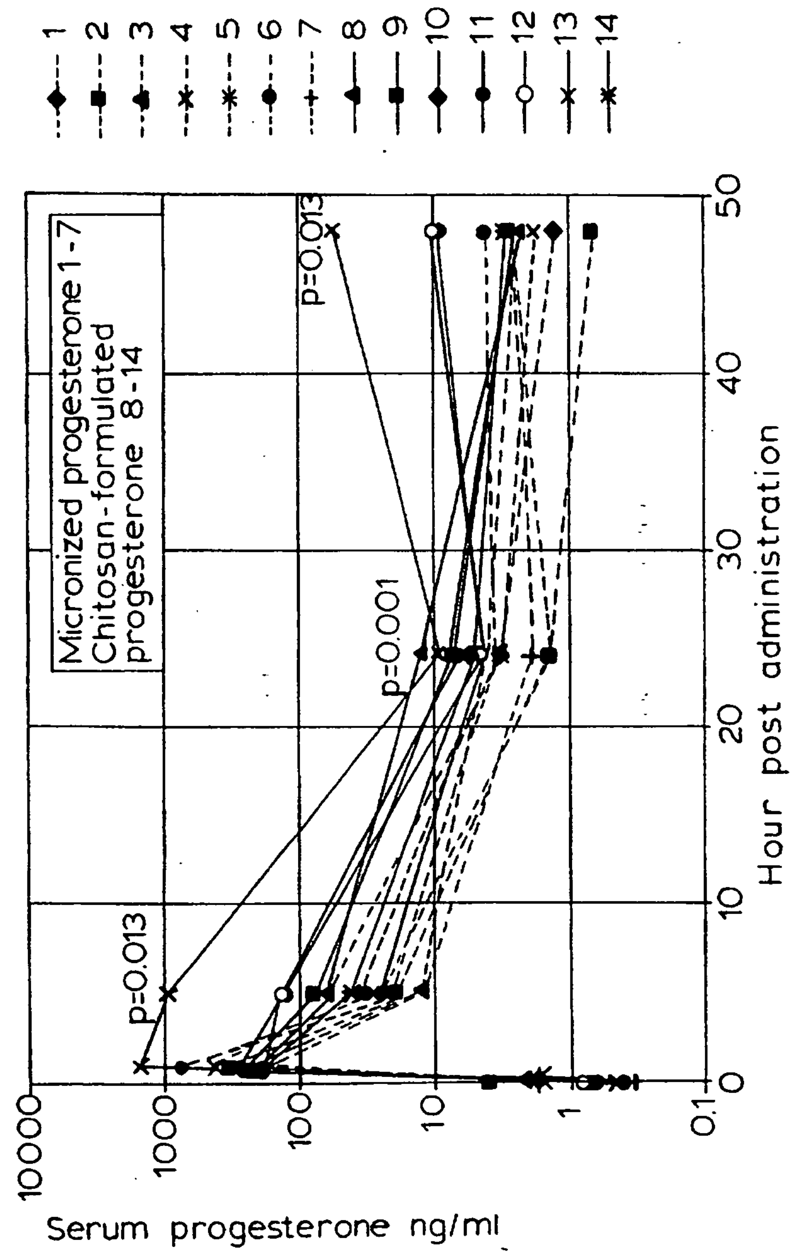
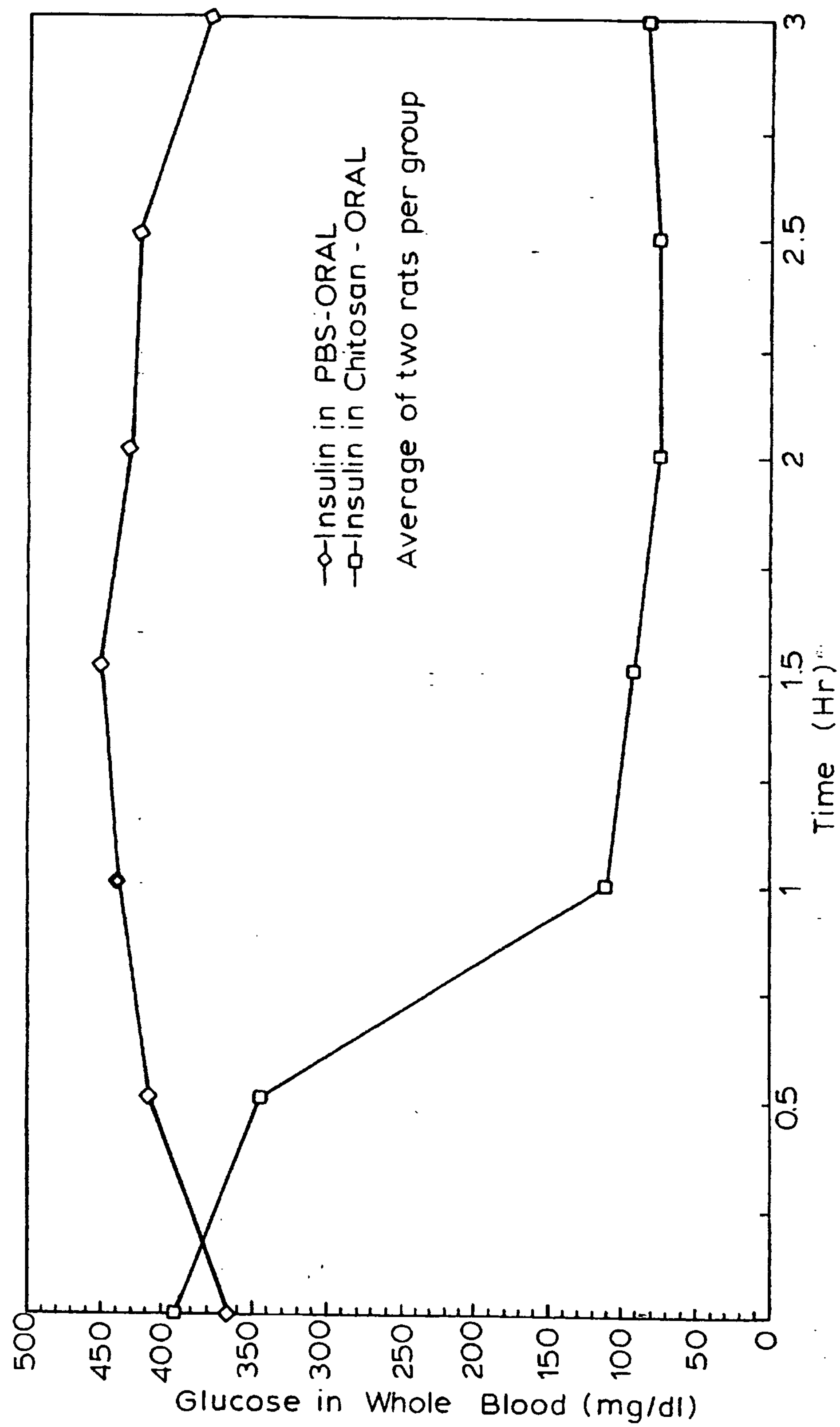

Micronized progesterone 1-7
Chitosan-formulated progesterone 8-14
p=0.013
p=0.001
p=0.013
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Serum progesterone ng/ml
10000
1000
100
10
1
0.1
Hour post administration
0
10
20
30
40
50

FIGURE 4


—◇—Insulin in PBS-ORAL
—□—Insulin in Chitosan - ORAL
Average of two rats per group
Glucose in Whole Blood (mg/dl)
Time (Hr)
500
450
400
350
300
250
200
150
100
50
0
0.5
1
1.5
2
2.5
3